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THE EFFECT OF CYCLIC GMP ON PHOSPHOFRUCTOKINASE FROM RAT TISSUES

RIVKA BEITNER, SHOSHANA HABERMAN and TOVA CYCOWITZ

Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel)

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Summary

In view of the recently proposed hypothesis of biologic regulation through opposing influences of cyclic AMP and cyclic GMP, and since cyclic AMP is a well-known allosteric activator of phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), the effect of cyclic GMP on the activity of this enzyme from several rat tissues was investigated. It was found that cyclic GMP exerted an inhibitory effect on the activity of rat heart and skeletal muscle phosphofructokinase. This effect was most pronounced under conditions in which the enzyme was partially inhibited by ATP or by citrate. Cyclic GMP also antagonized the deinhibitory action of cyclic AMP and other allosteric activators, such as glucose 1,6-bisphosphate or AMP, on the ATP or citrate-inhibited heart or muscle phosphofructokinase. In contrast to the heart and skeletal muscle phosphofructokinase, the adipose-tissue enzyme was not affected by cyclic GMP to any significant degree. The antagonistic action of cyclic GMP to the activation of heart-phosphofructokinase, may suggest a mechanism by which the activity of phosphofructokinase is synchronized with the activity of glycogen phosphorylase, as a result of acetylcholine action in heart, to achieve a decrease in total glycogenolysis and glycolysis.

Introduction

Guanosine 3',5'-cyclic monophosphate (cyclic GMP) research is only in its early stages of development and very little is known concerning its possible biochemical functions in cell metabolism. Recent reports (for reviews see Goldberg et al., refs. 1 and 2) have revealed that there are a number of biological systems in which cyclic AMP and cyclic GMP seem to impose antagonistic regulatory influences. Based on these reports, and since it has been well established [3—9] that cyclic AMP is a potent allosteric activator of mammalian phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), the

rate-limiting enzyme in glycolysis, we have undertaken an investigation of the effect of cyclic GMP on the activity of this enzyme from several rat tissues. The results reported here reveal that cyclic GMP exerts an inhibitory effect on the activity of rat heart and skeletal muscle phosphofructokinase, it also antagonizes the stimulatory action of cyclic AMP and other allosteric activators, on the activity of this enzyme. Rat adipose-tissue phosphofructokinase was found to be less sensitive to inhibition by cyclic GMP. The possible physiological significance of these results will be discussed.

Materials and Methods

Materials

Fructose 6-phosphate, ATP, NADH, aldolase, triosephosphate isomerase, α-glycerophosphate dehydrogenase, adenosine 3′,5′-cyclic monophosphate, guanosine 3′,5′-cyclic monophosphate, guanosine 5′-monophosphate and glucose 1,6-bisphosphate (free of fructose 1,6-bisphosphate, analysed by the method of Bücher and Hohorst [10]), were purchased from Sigma Chemical Company. AMP was obtained from Serva. Sodium citrate was from Baker Chemical Company. Imidazole was the low fluorescence grade from Sigma. DEAE-cellulose (Whatman DE11) was obtained from Whatman Biochemical Ltd.

Preparation of phosphofructokinase from rat tissues

Phosphofructokinase was purified from rat heart and skeletal muscle according to the procedure of Pogson and Randle [11]. About 3 g tissue (hearts or hind leg muscles from male albino rats, 100—150 g) was homogenized with 4 vols. of cold 50 mM Tris/2 mM EDTA (pH 8.0), in an ice bath, using a Willems-Polytron Homogenizer (Model PT 10ST "OD", Kinematica) at a setting of 3.5. The homogenate was centrifuged at 4° C for 30 min at 27 000 × *g* and the supernatant was added to the DEAE-cellulose column [11]. Activity was eluted with 0.3 M Tris/HCl buffer (pH 8.0). The eluate was collected in 5 fractions of 3 ml each, and the fractions with the highest activity (usually the second and third) were used for the experiments.

The purification of phosphofructokinase from epididymal fat pads, obtained from male albine rats (100—150 g), was conducted by a similar procedure, with the modifications described by Denton and Randle [5].

The specific activity of the phosphofructokinase preparations, measured under the conditions described by Massey and Deal [12] for assay of maximum activity (pH 8.0), was approximately 0.7, 10, and 12 unit/mg of protein, for the adipose tissue, heart, and skeletal muscle enzyme respectively (about 10—16-fold purification). A unit of activity is defined as the amount of enzyme catalyzing the formation of 1 μmol of fructose 1,6-bisphosphate per min at 25° C.

Protein was measured by the method of Lowry et al. [13] with crystalline bovine serum albumin as a standard.

Since storage led to loss of activity, the enzyme was freshly prepared each day.

Assay of phosphofructokinase activity

Phosphofructokinase activity was assayed by coupling with aldolase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase systems. The rate of disappearance of NADH was followed in a Perkin-Elmer (MPF-44) fluorescence spectrophotometer at 25°C. All coupling enzymes were dialysed to remove ammonium sulfate at 0°C against 0.01 M Tris · HCl buffer (pH 8.0). No SO_4^{2-} ion was detectable, by $BaCl_2$-HCl, in the solutions used.

The activity of heart or skeletal muscle phosphofructokinase was measured under conditions similar to those described by Massey and Deal [12] for assay of allosteric activity (pH 7.0); 1—2 μl of rat heart or skeletal muscle phosphofructokinase preparation was added to the reaction mixture which contained in a final volume of 1 ml: 50 mM imidazole buffer (pH 7.0), 5 mM $MgCl_2$, 50 mM KCl, 50 mM 2-mercaptoethanol, 100 $\mu g \cdot ml^{-1}$ aldolase (dialysed), 5 $\mu g \cdot ml^{-1}$ triosephosphate isomerase (dialysed), 50 $\mu g \cdot ml^{-1}$ α-glycerophosphate dehydrogenase (dialysed), 15 μM NADH, ATP, and other additions as specified in text, figures or tables. After 2 min the reaction was initiated by addition of fructose 6-phosphate (at concentrations given in figures or tables).

The activity of rat adipose-tissue phosphofructokinase was measured under conditions similar to those described by Denton and Randle [5]; 25 μl of the phosphofructokinase preparation were added to the reaction mixture which contained in a final volume of 1 ml: 20 mM imidazole buffer (pH 7.4), 0.01% bovine plasma albumin 5 mM $MgCl_2$, 150 mM potassium acetate, 30 $\mu g \cdot ml^{-1}$ aldolase (dialysed), 1 $\mu g \cdot ml^{-1}$ triosephosphate isomerase (dialysed), 10 $\mu g \cdot ml^{-1}$ α-glycerophosphate dehydrogenase (dialysed), 15 μM NADH, ATP and other additions as specified in text, figures or tables. After 1.5 min the reaction was initiated by addition of fructose 6-phosphate.

Controls were recorded for each experiment in which fructose 6-phosphate was omitted.

In all these experiments cyclic GMP was always added before the addition of the other modulators of the enzyme.

Results

The influence of cyclic GMP on rat heart phosphofructokinase

The effect of cyclic GMP on rat heart phosphofructokinase activity was tested under various assay conditions. The results summarized in Table I show that cyclic GMP inhibited the enzyme, in a concentration dependent manner, at both non-inhibitory concentration of ATP (0.1 mM), as well as under conditions in which the enzyme was partially inhibited by ATP (0.25 mM) or by citrate. The ATP or citrate-inhibited enzyme was found to be more susceptible to inhibition by low concentrations of cyclic GMP than the non-inhibited enzyme. It can be seen that the enzyme was inhibited by the cyclic nucleotide at its physiological concentrations [14,15].

The ability of cyclic GMP to antagonize the deinhibitory action of cyclic AMP, AMP or glucose-1,6-bisphosphate, on the ATP or citrate-inhibited heart phosphofructokinase, was tested. Figs. 1 and 2 show that the activity of the ATP-inhibited rat heart phosphofructokinase, stimulated by variable cyclic AMP (Fig. 1), or AMP (Fig. 2) concentrations, was markedly reduced in the

TABLE I

THE INHIBITORY EFFECT OF CYCLIC GMP ON THE ACTIVITY OF RAT HEART PHOSPHOFRUCTOKINASE

The Fru-6-*P* concentration in the assay medium was 0.21 mM. Rat heart phosphofructokinase preparation was added at a concentration of about 1.9 μg protein per ml. Other experimental conditions were as described under Materials and Methods. Values are means ± S.E. for 6 experiments. (cGMP, guanosine 3',5'-cyclic monophosphate)

Additions	Phosphofructokinase activity (nmol Fru-1,6-P_2/min per ml)	Inhibition by cGMP (%)
ATP (0.1 mM)	4.20 ± 0.06	
ATP (0.1 mM) + cGMP (0.01 μM)	4.16 ± 0.09	0
ATP (0.1 mM) + cGMP (0.05 μM)	3.78 ± 0.04	10
ATP (0.1 mM) + cGMP (0.5 μM)	2.39 ± 0.04	43
ATP (0.1 mM) + cGMP (1 μM)	2.14 ± 0.06	49
ATP (0.25 mM)	2.22 ± 0.07	
ATP (0.25 mM) + cGMP (0.01 μM)	1.99 ± 0.02	22
ATP (0.25 mM) + cGMP (0.05 μM)	1.40 ± 0.04	37
ATP (0.25 mM) + cGMP (0.5 μM)	1.20 ± 0.06	46
ATP (0.25 mM) + cGMP (1 μM)	0.69 ± 0.01	69
ATP (0.1 mM) + citrate (0.05 mM)	3.15 ± 0.04	
ATP (0.1 mM) + citrate (0.05 mM) + cGMP (0.01 μM)	2.31 ± 0.04	27
ATP (0.1 mM) + citrate (0.05 mM) + cGMP (0.05 μM)	1.89 ± 0.04	40
ATP (0.1 mM) + citrate (0.05 mM) + cGMP (0.5 μM)	1.48 ± 0.08	53

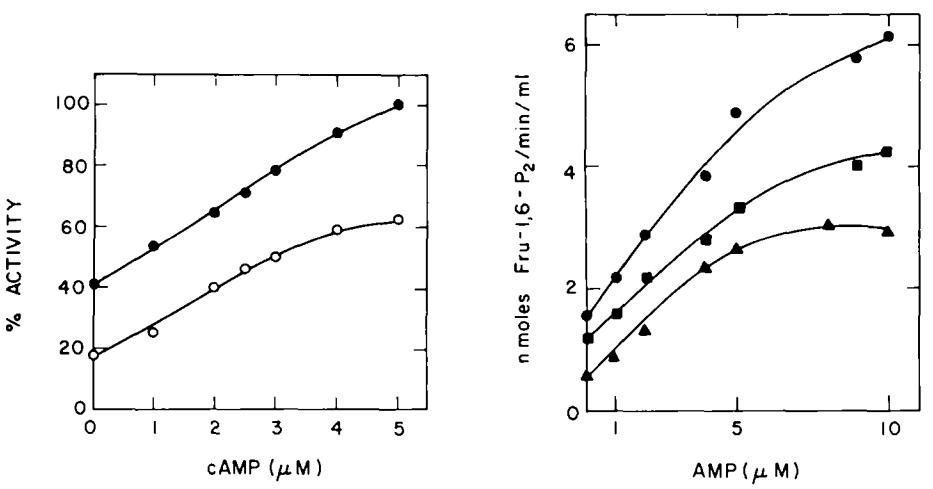


Fig. 1. The inhibitory effect of cyclic GMP on the activity of rat heart phosphofructokinase at variable cyclic AMP concentrations. Assays were carried out at inhibitory ATP concentration (0.4 mM), either in the absence of cyclic GMP (●), or in the presence of 1 μM cyclic GMP (○), at variable cyclic AMP concentrations. Phosphofructokinase activity is expressed as percent of the activity measured at non-inhibitory concentration of ATP (0.1 mM). 100% phosphofructokinase activity refers to 4.2 nmol of Fru-1,6-P_2 formed in 1 ml of the reaction mixture within 1 min. Other experimental conditions were as described in Table I.

Fig. 2. The inhibitory effect of cyclic GMP on the activity of rat heart phosphofructokinase at variable AMP concentrations. Assays were carried out at inhibitory ATP concentration (0.5 mM), either in the absence of cyclic GMP (●), or in the presence of cyclic GMP, at 0.5 μM (■) and 1 μM (▲). All reaction mixtures contained 0.1 mM Fru-6-*P* and about 3.3 μg protein/ml of the rat heart phosphofructokinase preparation. Other reactants and conditions of assay were as described under Materials and Methods.

presence of cyclic GMP. The experiments demonstrated in Fig. 3 reveal that cyclic GMP also antagonized the deinhibitory action of glucose 1,6-bisphosphate on the ATP-inhibited enzyme, in a concentration dependent manner. This effect was readily demonstrated at cyclic GMP concentrations as low as 0.01 μM. Similar experiments were carried out on the citrate-inhibited heart phosphofructokinase (Table II, Fig. 4). The results summarized in Table II show that cyclic GMP antagonized the deinhibitory action of glucose 1,6-bisphosphate or AMP on the citrate-inhibited heart enzyme. The effect induced by cyclic GMP increased by raising its concentration. Under similar conditions cyclic GMP was also found to antagonize the deinhibitory action of cyclic AMP on the citrate-inhibited heart phosphofructokinase, in a concentration dependent manner (Fig. 4).

The influence of cyclic GMP on skeletal muscle phosphofructokinase

The effect of cyclic GMP on rat skeletal muscle phosphofructokinase was found to be similar to that induced on the heart enzyme. Fig. 5 shows the effect of cyclic GMP on the skeletal muscle enzyme, measured at variable cyclic GMP concentrations and with two concentrations of ATP: non-inhibitory (0.025 mM) and strongly inhibitory (0.25 mM). It can be seen that cyclic GMP exerted an inhibitory effect on the non-inhibited enzyme. This effect, however, was relatively small when compared to its antagonistic action to the activation of the ATP-inhibited enzyme by cyclic AMP. Cyclic GMP also antagonized the deinhibitory action of glucose 1,6-bisphosphate (Fig. 6) or AMP (not shown) on the ATP-inhibited enzyme. Similar results were obtained on the citrate-in-

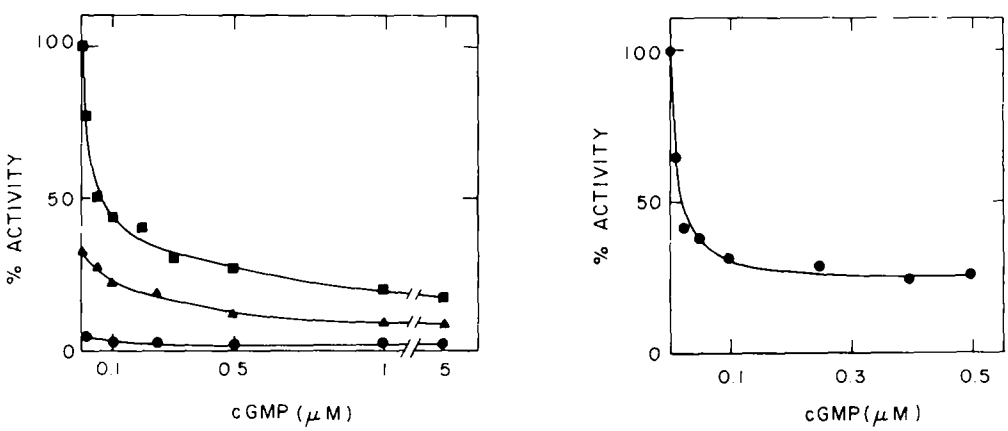


Fig. 3. The antagonistic action of cyclic GMP to the activation of rat heart phosphofructokinase by Glc-1,6-P_2. Assays were carried out either in the absence of Glc-1,6-P_2 (●), or in the presence of Glc-1,6-P_2, at 10 μM (▲) and 25 μM (■), at variable cyclic GMP concentrations. All reaction mixtures contained 40 μM Fru-6-P, an inhibitory ATP concentration (0.1 mM), and 1.9 μg protein/ml of the rat heart phosphofructokinase preparation. 100% activity refers to 4.62 nmol of Fru-1,6-P_2 formed per min per ml. Other experimental conditions were as described under Materials and Methods.

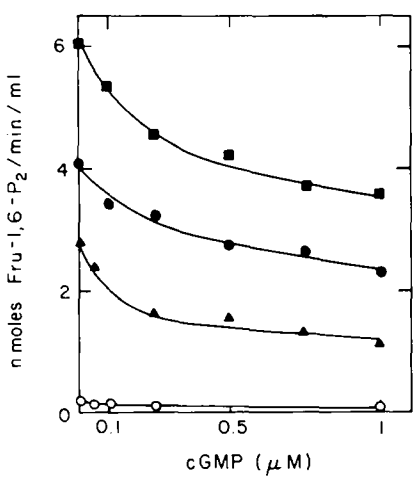
Fig. 4. The antagonistic action of cyclic GMP to the activation of the citrate-inhibited rat heart phosphofructokinase by cyclic AMP. Assays were carried out under the same experimental conditions as described in Table II, in the presence of 0.25 mM citrate and 5 μM cyclic AMP, at variable cyclic GMP concentrations. 100% activity refers to 2.58 nmol of Fru-1,6-P_2 formed per min per ml.

TABLE II

THE ANTAGONISTIC ACTION OF CYCLIC GMP TO THE ACTIVATION OF THE CITRATE-INHIBITED RAT HEART PHOSPHOFRUCTOKINASE BY AMP OR BY Glc-1,6-P_2

The reaction mixtures contained: 0.2 mM Fru-6-*P*, 0.1 mM ATP, and about 1.9 μg protein/ml of the rat heart phosphofructokinase preparation. Other reactants and conditions of assay were as described under Materials and Methods. Values are means ± S.E. for 6 experiments. (cGMP, guanosine 3',5'-cyclic monophosphate)

Additions	Phosphofructokinase activity (nmol Fru-1,6-P_2/min per ml)	Inhibition by cGMP (%)
None	4.20 ± 0.06	
Citrate (0.25 mM)	0.86 ± 0.01	
Citrate (0.25 mM) + AMP (5 μM)	2.60 ± 0.05	
Citrate (0.25 mM) + AMP (5 μM) + cGMP (0.01 μM)	1.98 ± 0.07	24
Citrate (0.25 mM) + AMP (5 μM) + cGMP (0.05 μM)	1.4 ± 0.04	46
Citrate (0.25 mM) + AMP (5 μM) + cGMP (0.5 μM)	1.26 ± 0.01	62
Citrate (0.25 mM) + Glc-1,6-P_2 (7.5 μM)	2.94 ± 0.08	
Citrate (0.25 mM) + Glc-1,6-P_2 (7.5 μM) + cGMP (0.01 μM)	2.32 ± 0.09	21
Citrate (0.25 mM) + Glc-1,6-P_2 (7.5 μM) + cGMP (0.05 μM)	1.72 ± 0.05	42
Citrate (0.25 mM) + Glc-1,6-P_2 (7.5 μM) + cGMP (0.5 μM)	1.35 ± 0.07	54



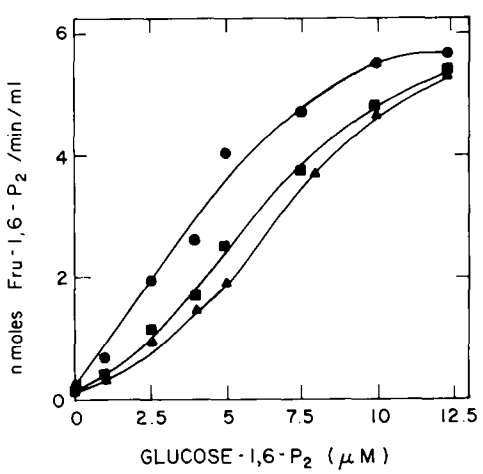


Fig. 5. The influence of cyclic GMP on rat skeletal muscle phosphofructokinase. Assays were carried out either at non-inhibitory ATP concentration (0.025 mM) (●), or at inhibitory ATP concentration (0.25 mM), in the absence of cyclic AMP (○), and in the presence of cyclic AMP, at 5 μM (▲) and 10 μM (■), at variable cyclic GMP concentrations. The reaction mixtures contained 0.08 mM Fru-6-*P* and 2.4 μg protein/ml of the rat skeletal muscle phosphofructikinase preparation. Other assay conditions were as described under Materials and Methods.

Fig. 6. The antagonistic action of cyclic GMP to the activation of rat skeletal muscle phosphofructokinase by Glc-1,6-P_2. Assays were carried out at inhibitory ATP concentration (0.25 mM), either in the absence of cyclic GMP (●), or in the presence of cyclic GMP, at 0.5 μM (■) and 1 μM (▲), at variable Glc-1,6-P_2 concentrations. The reaction mixtures contained 0.08 mM Fru-6-*P* and 2.4 μg protein/ml of the rat skeletal muscle phosphofructokinase preparation. Other conditions were as described under Materials and Methods.

hibited enzyme (not shown). Thus, in all respects the behaviour of the rat skeletal muscle phosphofructokinase resembled that of the heart enzyme.

Similar experiments were performed on highly purified rabbit muscle phosphofructokinase (Sigma). The results of these experiments are summarized in Table III. It can be seen that the influence of cyclic GMP on the activity of this highly purified muscle enzyme was similar to that found with the less purified rat muscle or heart phosphofructokinase preparations; cyclic GMP exerted an inhibitory effect on the activity of this enzyme and it also antagonized the deinhibitory action of cyclic AMP, glucose 1,6-bisphosphate or AMP, on the ATP or citrate-inhibited phosphofructokinase. Table III also reveals the specificity of the effect of cyclic GMP, since GMP under identical experimental conditions had no effect on the activity of the enzyme.

The influence of cyclic GMP on rat adipose-tissue phosphofructokinase

In contrast to the heart and skeletal muscle phosphofructokinase, the adipose-tissue enzyme was not affected by cyclic GMP to any significant degree. The results summarized in Table IV show that cyclic GMP exerted only a very small inhibitory effect on the activity of adipose-tissue phosphofructokinase, measured either at non-inhibitory ATP concentration (0.025 mM), or under conditions in which the enzyme was partially inhibited by ATP (0.15 mM) or by citrate. The ability of cyclic GMP to antagonize the deinhibitory action of cyclic AMP, AMP, or glucose 1,6-bisphosphate, on the ATP or citrate-inhibited

TABLE III

THE INFLUENCE OF CYCLIC GMP ON HIGHLY PURIFIED RABBIT MUSCLE PHOSPHOFRUCTOKINASE

Crystalline rabbit muscle phosphofructokinase, obtained from Sigma Chemical Co. (Lot 102C-8721-9, type III, specific activity 175 units/mg protein), was charcoal-treated and dialyzed by the procedure described by Bock and Frieden [44]. Assays were carried out at inhibitory ATP concentration (0.42 mM) (A), or at inhibitory citrate concentration (0.4 mM) (B). The concentration of ATP in B was 0.08 mM. The reaction mixtures contained: 88 μM Fru-6-*P*, 45 μM NADH and 0.6 μg protein/ml of the rabbit muscle phosphofructokinase. Other assay conditions were as described under Materials and Methods. 100% activity in A refers to 3 nmol of Fru-1,6-P_2 formed per min per ml. 100% activity in B refers to 2.8 nmol of Fru-1,6-P_2 formed per min per ml. (cGMP, guanosine 3′,5′-cyclic monophosphate; cAMP, adenosine 3′,5′-cyclic monophosphate.

Additions	Phosphofructokinase activity (%)	
	A	B
None	100	100
cGMP (0.05 μM)	75	72
cGMP (0.5 μM)	64	54
cGMP (1 μM)	41	38
GMP (0.05 μM)	100	100
GMP (0.5 μM)	100	100
GMP (1 μM)	100	100
cAMP (10 μM)	473	498
cAMP (10 μM) + cGMP (0.5 μM)	394	326
AMP (5 μM)	333	344
AMP (5 μM) + cGMP (0.5 μM)	199	185
Glc-1,6-P_2 (5 μM)	692	680
Glc-1,6-P_2 (5 μM) + cGMP (0.5 μM)	462	436

TABLE IV

THE INFLUENCE OF CYCLIC GMP ON RAT ADIPOSE TISSUE PHOSPHOFRUCTOKINASE

The reaction mixtures contained: 0.32 mM Fru-6-*P*, and about 25 μg/ml of the rat adipose-tissue phosphofructokinase preparation. Other conditions were as described under Materials and Methods. Values are means ± S.E. for 6 experiments. (cGMP, guanosine 3′,5′-cyclic monophosphate.)

Additions	Phosphofructokinase activity (nmol Fru-1,6-P_2/min per ml)	Inhibition by cGMP (%)
ATP (0.025 mM)	0.48 ± 0.07	
ATP (0.025 mM) + cGMP (0.05 μM)	0.48 ± 0.06	0
ATP (0.025 mM) + cGMP (0.1 μM)	0.43 ± 0.08	10
ATP (0.025 mM) + cGMP (0.5 μM)	0.42 ± 0.05	12
ATP (0.025 mM) + cGMP (1 μM)	0.43 ± 0.03	10
ATP (0.15 mM)	0.22 ± 0.08	
ATP (0.15 mM) + cGMP (0.05 μM)	0.22 ± 0.04	0
ATP (0.15 mM) + cGMP (0.1 μM)	0.20 ± 0.02	9
ATP (0.15 mM) + cGMP (0.5 μM)	0.19 ± 0.04	14
ATP (0.15 mM) + cGMP (1 μM)	0.18 ± 0.07	18
ATP (0.025 mM) + citrate (0.25 mM)	0.33 ± 0.02	
ATP (0.025 mM) + citrate (0.25 mM) + cGMP (0.05 μM)	0.33 ± 0.08	0
ATP (0.025 mM) + citrate (0.25 mM) + cGMP (0.1 μM)	0.30 ± 0.11	9
ATP (0.025 mM) + citrate (0.25 mM) + cGMP (0.5 μM)	0.30 ± 0.07	9
ATP (0.025 mM) + citrate (0.25 mM) + cGMP (1 μM)	0.28 ± 0.03	15

enzyme, was also tested, and found to be very poor. It should be noted that the adipose-tissue phosphofructokinase was very sensitive to activation by glucose 1,6-bisphosphate. Thus, in this respect its behaviour is similar to that of phosphofructokinase from other animal tissues [7,16,17]. The regulatory role of glucose 1,6-biphosphate in glucose metabolism was pointed out in our previous studies [18—21].

Discussion

Goldberg et al. [1] have recently proposed the hypothesis of biologic regulation through opposing influences of cyclic GMP and cyclic AMP, which they have named the "Yin-Yang Hypothesis". The results of the present experiments add new support to this hypothesis by demonstrating the inhibitory action of cyclic GMP on the activity of rat heart and skeletal muscle phosphofructokinase, opposing the activatory action of cyclic AMP, and other allosteric activators, on this enzyme.

Cyclic GMP has been implicated as a mediator of cholinergic receptor activity in various tissues [1,2,14,15,22—26]. In the isolated perfused rat heart it was demonstrated that acetylcholine caused a significant elevation in myocardial cyclic GMP levels, which was associated with suppression of cardiac contractility [1,14,22,27] Acetylcholine was also shown to be capable of inhibiting the glycogenolytic effect of epinephrine on the hearts [28,29], and to antagonize the activation of glycogen phosphorylase by epinephrine [27,29]. Mansour [7] has pointed out to a synchronous activation of glycogen phosphorylase and phosphofructokinase following epinephrine administration,

through which an increase in total glycogenolysis and glycolysis is achieved. Similarly, the antagonistic action of cyclic GMP to the activation of heart phosphofructokinase, demonstrated in the present paper, may suggest a mechanism by which the activity of phosphofructokinase is synchronized with the activity of glycogen phosphorylase as a result of acetylcholine action.

The present experiments reveal that adipose-tissue phosphofructokinase, in contrast to the heart and muscle enzyme, is not affected by cyclic GMP to any significant degree. There is suggestive evidence that cyclic GMP plays a role in the action of insulin in fat cells [30,31]; in these cells insulin, like cholinergic drugs, has been shown to be capable of elevating cyclic GMP levels. If cyclic GMP is associated with the actions of insulin in the adipose-tissue, it is unlikely that it would inhibit the adipose tissue phosphofructokinase activity, in view of the stimulatory action of insulin on glucose oxidation via the Embden-Meyerhof pathway in this tissue [32]. The insensitivity of the adipose-tissue phosphofructokinase to inhibition by cyclic GMP may be due to the presence of different isozymes of phosphofructokinase in this tissue [33,34]; whereas skeletal muscle and heart contain a single and identical phosphofructokinase isozyme [34—36], in adipose tissue five isozymes (a muscle-liver hybrid set) have been found [33,34].

The possible physiological significance of the inhibitory effect of cyclic GMP on skeletal muscle phosphofructokinase is not clear. This effect cannot be related to the action of insulin, since it has been reported [37,38] that the levels of cyclic GMP in muscle are not altered by insulin. Our earlier studies [39] have also revealed that insulin stimulates the phosphofructokinase in muscle. The cyclic GMP effect on the muscle phosphofructokinase cannot also be related to acetylcholine, since acetylcholine [2] or its analog, carbachol [38], did not cause a rise in the cyclic GMP is muscle. The increase in the concentration of cyclic GMP brought about by acetylcholine in various tissues, has been attributable to its interaction with muscarinic, but not with nicotinic receptors [23]. Little is known about the factors that regulate the concentration of cyclic GMP in the skeletal muscle. Steiner et al. [40] have implicated glucocorticoids as agents which can suppress cyclic GMP levels in skeletal muscle. It is possible that some other, as yet unidentified agent, may increase cyclic GMP concentration in this tissue, leading to inhibition of phosphofuctokinase.

In contrast to skeletal muscle, the effects of cyclic GMP on heart phosphofructokinase, demonstrated in the present paper, may be related to the actions of acetylcholine in this tissue. Acetylcholine causes a rise in the cyclic GMP in heart [14,15] to levels which are within the concentration range that would affect phosphofructokinase. It has been suggested [1] that cyclic GMP is involved in suppressing and cyclic AMP with facilitating cardiac contractility. It is possible that this process may also be linked to the glycogenolytic and glycolytic processes, through a synchronous regulation of glycogen phosphorylase and phosphofructokinase, by the two cyclic nucleotides, acting in opposition to one another, to meet the energy demand of the cell.

Recently a new regulatory mechanism of mammalian phosphofructokinase activity by phosphorylation-dephosphorylation has been described [41—43]. The interconversion reactions, however, were found not to be influenced by cyclic AMP or cyclic GMP [43]. Thus, in contrast to the complexity of the

phosphorylase regulatory system, the cyclic nucleotides seem to act directly on phosphofructokinase, in opposition to one another.

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